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Remarks:

Each of dependent claims 148, 185, 188, 189, 190, 194, and 196 were indicated as containing allowable subject matter in the first office action on the merits. Paper No. 11. These claims were amended to put them in independent format to put them in condition for issuance. 2 October paper. All other claims were cancelled in order to expedite the issuance of the allowable claims. 2 October paper. However, the non-final office action dated December 29, 2003, rejected the formerly allowable claims over prior art. Paper No. 1203. By this amendment Applicants now add back the claims that were canceled (renumbering them as indicated in the table below). Applicants also put the formerly allowable claims back into dependent format to facilitate their grouping with other claims sharing common recitations.

Old claim number	New claim number
1	219
132-147	220-235
149-167	236-254
182-184	255-257
186-187	258-259
191-193	260-262
195	263
199-204	264-269

In addition, the following recitations have been added to the claims:

- "culturing a 3-dimensional mass of living cells to form a proteinaceous extracellular matrix surrounding said cells" (claim 219)
- "decellularizing the proteinaceous extracellular matrix" (claim 219)
- "tissue engineered construct comprising a 3-dimensional proteinaceous extracellular matrix" (claim 219)
- "tissue engineered construct comprising a 3-dimensional proteinaceous extracellular matrix" (claim 220)

- "whereby a proteinaceous extracellular matrix surrounding said cells is formed" (claims 220 and 255)
- "a growth period of about 6 to 8 weeks" (claim 221)
- "a tissue engineered construct having a thickness of > 200 μm"(claim 222)
- "construct comprising a 3-dimensional proteinaceous extracellular matrix synthesized by a first population of cells grown *in vitro* on a substrate"(claim 255)

The specification amply supports the recitations added by amendment to the claims. Supportive portions of the specification are directly quoted below, with emphasis added. The 3-dimensional mass of living cells is supported at paragraph 52[1] which states:

> "Tissue Engineered Construct
> "This term is generally used herein to refer to a two or **three dimensional** mass of living mammalian tissue produced primarily by growth in vitro. The construct may include one or more types of tissue, and each tissue may include one or more types of **cells**."

Formation of a 3-dimensional matrix is supported at paragraph 81, which states:

> "These references disclose techniques for establishing a **three-dimensional matrix**, inoculating the matrix with the desired cells, and maintaining the culture. In general, a tissue engineered construct is produced by seeding cells onto an appropriate substrate and culturing the cells under conditions suitable for growth."

A growth period of about 6 to 8 weeks is supported at paragraph 68 and at paragraph 132, which state:

> "For example, in certain embodiments of the invention, to produce a tissue engineered blood vessel, a substrate is seeded with smooth muscle cells and cultured for a period of time**, e.g., 6 weeks**. After this first growth period the construct is seeded with endothelial cells and cultured for a further growth period, e.g., 1-2 weeks. "

> "The substrate is cultured with the application of pulsatile stretch for approximately **6-8 weeks**, during which a substantial proteinaceous extracellular matrix is secreted, and the construct attains desired physical properties and thickness."

[1] Paragraph numbers refer to the published application.

A tissue engineered construct having a thickness of >200 μm, is supported at Fig. 6A which shows a cross-section of a construct with a ruler bar of 200 μm. The construct is clearly larger than the ruler bar. See also paragraph 17, which states:

> "After the cells have formed a tissue of the **desired thickness**, the construct is decellularized."

The proteinaceous nature of the extracellular matrix is taught at paragraph 67, which teaches:

> "As the cells grow and divide on and/or in the substrate they secrete **extracellular matrix proteins** such as collagen and elastin. The construct is cultured for a period of time sufficient to produce a construct of desired thickness and/or properties, **consisting primarily of secreted proteins** and cells."

Growth on a substrate by a first population of cells is taught at paragraph 67, which states:

> "Typically these methods involve **seeding (i.e., contacting) a substrate with cells and culturing the seeded substrate under conditions suitable for growth of the cells** to form a tissue engineered construct."

Formation of a proteinaceous extracellular membrane surrounding the cells is evidenced in Fig. 5A which shows the matrix surrounding the cells prior to decellularization. Fig. 5B shows the matrix after decellularization.

Other amendments are merely cosmetic or formal, perfecting antecedent basis and otherwise clarifying claim language.

The rejection of claim 148 under 35 USC § 102(b)

Claim 148 is rejected as anticipated by Bruchman WO 95/29712 ('712). This rejection is respectfully traversed.

To reject a claim as anticipated, each and every element as set forth in the claim must be either expressly or inherently described in a single prior art reference. *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 U.S.P.Q.2d (BNA) 1051, 1053 (Fed. Cir. 1987).

Bruchman is cited as teaching the same general type of tissue engineered construct as described in claim 148. The Office Action concedes, however, that Bruchman does not teach the electrical stimulation (product-by-process limitation) that is recited in claim 148. Nonetheless, the PTO makes the rejection because the properties of the tissue engineered construct that are imparted by the electrical stimulation are not explicit or apparent.

Claim 148 is dependent on claim 221 which is dependent on claim 220. Claim 220 recites that the tissue engineered construct comprises "a 3-dimensional proteinaceous extracellular matrix." Claim 220 further recites that the matrix is formed "surrounding said cells." Bruchman does not teach such a matrix.

Bruchman teaches the growth of a monolayer of endothelial cells grown on a substrate layer of smooth muscle cells. Page 6, lines19-32. Bruchman teaches that the endothelial cells lay down a subendothelium matrix between the endothelial cell layer and the smooth muscle cell layer. Page 6, lines 27-30. The subendothelial matrix is said to be $\leq$ 1 μm. Page 14, line 6. Thus Bruchman teaches a very thin layer of matrix which forms between two cell layers. This thin layer of matrix is not a 3-dimensional proteinaceous extracellular matrix that forms surrounding cells as required by claim 148. This thin layer is a 2-dimensional layer which forms below a layer of cells.

Claim 221 also recites a growth period of 6-8 weeks. Bruchman teaches a growth period of only 10 days. Page 14, line 3. Thus Bruchman does not teach this feature of the claimed invention.

Thus Bruchman does not teach each and every limitation of claim 220, claim 221, or claim 148. The rejection for anticipation should therefore be withdrawn.

The rejection of claims 148, 185, 189, 190, 194, 196, and 197 under 35 USC §102(b)

Claims 148, 185, 189, 190, 194, 196, and 197 stand rejected as anticipated by either of Bruchman (1997) WO 97/46266 ('266) or Bruchman (1999) U.S. 5,879,383 ('383). This rejection is respectfully traversed.

As discussed above, the PTO asserts that the recitation in each of claims 148 and 185 regarding electrical stimulation does not impart a distinct property on the claimed product. Similarly, the PTO does not find apparent the difference imparted to the product by the recitation in claims 189 and 197 of use of neonatal cells, by the recitation in claim 194 of using *in vitro* cultured cells, by the recitation in claim 190 of using genetically transformed cells, and by the recitation of seeding with two different cell types in claim 196.

While applicant does not concede that these recitations would impart no functional or structural properties to the claimed constructs that would distinguish them from the constructs taught by Bruchman, applicant points out that there are other recitations which do distinguish the claimed constructs from Bruchman's.

Claim 148 is dependent on claim 221, which is dependent on 220, and is discussed above. Claims 185, 189, 190, 194, 196, and 197 are each dependent on claim 255. The recitations of claim 220 as discussed above include that the tissue engineered construct comprises "a 3-dimensional proteinaceous extracellular matrix" and that the matrix is formed "surrounding said cells." Claim 255 shares these recitations.

Bruchman's teachings in the applied '266 and '383 references are directed to the same type of tissue engineered constructs as in Bruchman's '712 reference. Briefly, Bruchman teaches a subendothelial matrix which is a thin layer formed between two cell monolayers. This layer is a 2-dimensional structure rather than the 3-dimensional structure formed by the present invention when an extracellular matrix forms surrounding cells rather than beneath a layer of cells. The cells of the present invention are not grown in monolayers, but rather in multilayered cell masses. After decellularization a honeycomb-like structure remains outlining and surrounding the spaces formerly occupied by the cells.

Because neither Bruchman '266 nor Bruchman '383 teach the type of construct claimed in independent claims 220 and 255, perforce they do not teach the constructs of dependent claims 148, 185, 189, 190, 194, 196, or 197.

Withdrawal of this rejection is respectfully requested because the applied references fail to teach all elements of the rejected claims.

The rejection of claims 188 and 198 under 35 USC §103(a)

Claims 188 and 198 are rejected as obvious over Bruchman '266 and Bruchman '383. This rejection is respectfully traversed.

> To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

MPEP § 2143. The rejection fails to make a prima facie case because the references do not teach or suggest all the claim limitations.

Claims 188 and 198 are directed to tissue engineered constructs made using two populations of cells. A first population of cells is used to make a decellularized matrix and a second population of cells is seeded on the decellularized matrix. The first population of cells (claim 188) or the second population of cells (claim 198) is specified as being human cells.

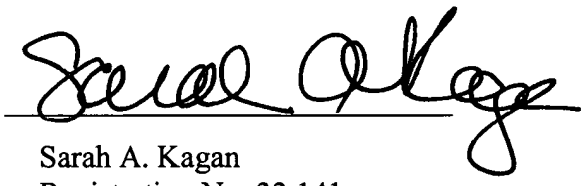
Claims 188 and 198 both depend from claim 255, thus they include all the limitations of claim 255. Claim 255 recites in part: "a decellularized tissue engineered construct comprising a 3-dimensional proteinaceous extracellular matrix synthesized by a first population of cells grown *in vitro* on a substrate whereby a proteinaceous extracellular matrix surrounding said cells is formed."

As discussed above, neither Bruchman '266 nor Bruchman '383 teaches such a construct. Bruchman teaches a construct which is a 2-dimensional matrix layer laid down between two cellular monolayers. The matrix is described as subendothelial because it forms below the endothelial layer. In contrast, the claimed constructs comprise a 3-dimensional proteinaceous extracellular matrix which is formed surrounding cells in a 3-dimensional mass of living cells. Bruchman's two applied references do not teach or suggest such a construct.

Withdrawal of the rejection and allowance of all claims is respectfully requested.

Respectfully submitted,

Dated: May 28, 2004

By: 

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